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IMPORTANCE OF THE FIRST C-TERMINAL RESIDUE OF DIBASIC CLEAVAGE SITES IN PRECURSOR PROTEOLYTIC PROCESSING. N. Brakch[1], M. Rholam[2] and P. Cohen[2]. [1]Division d' hypertension CHUV 1011 Lausanne Suisse. [2]Biochimie des Signaux Régulateurs Cellulaires et Moléculaire, Université Pierre et Marie Curie, Unité de Recherche Associée au CNRS, Paris France.

The selective pressure exerted on the evolution of hormone precursors and proproteins has led to the high conservation of basic residues as cleavage sites. However, examination of these sequences clearly indicate that whereas a number of the potential basic sites are indeed cleaved in vivo a significant percentage of these loci remain unprocessed. In an effort toward identification of other possible conserved residues in cleaved sites the amino acid sequences flanking 352 dibasic moieties (83 prohormones and pro-proteins) were examined. Frequency calculations on the occurrence of given residues at positions P_6 to P'_4 allowed us to delineate a number of features that might be in part responsible for discrimination between cleaved and uncleaved dibasic sites. Some amino acid occupy preferentially certain precursor subsites Met in P6 and P3, Asp and Ala in P'1, Pro in P6, Gly in P3 and P'2. In P'1 position the β-carbon branched side chain residues (Thr, Val, Leu, Ile) and Pro, Cys Met and Trp were either totally excluded or poorly represented, suggesting that they might be unfavourable to cleavage. The biological relevance of these observations was in vitro tested using both pro-oxcytocin convertase and Kex2 protease action on a series of pro-oxcytocin related synthetic substrates reproducing the Pro7-Leu15 sequence of the precursor in which the Ala13 residue (P'1 in the LysArg-Ala motif) was replaced by various amino acid residues. A good correlation was obtained on this model system indicating that P'1 residue of precursor dibasic processing sites is an important feature.

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DEVAZEPIDE, A CCK-A RECEPTOR ANTAGONIST, ATTENUATES THE EXPRESSION OF SENSITIZATION TO THE BEHAVIOURAL ACTIVATING EFFECTS OF AMPHETAMINE. N.J. DeSousa[1], G.R. Wunderlich[1], and F.J. Vaccarino[1,2]. Departments of Psychology[1] and Psychiatry[2], University of Toronto, Toronto, Canada, M5S 1A1 and Clarke Institute of Psychiatry, Toronto, Canada, M5T 1R8.

Numerous lines of evidence suggest that dopamine (DA) release from terminals in the nucleus accumbens is a critical substrate for locomotor activation. The present studies investigated the possibility that endogenous cholecystokinin (CCK), acting via CCK-A receptors, modulates the expression of DA-mediated locomotion produced by either circadian (exp. 1) or pharmacological (exps. 2 and 3) manipulations. In exp. 1, animals received counterbalanced i.p. injections of the CCK-A receptor antagonist devazepide (0.001, 0.01, and 0.1 mg/kg) or vehicle during either their light- (LP) or dark-phase (DP), 30 min after which their activity was monitored. In exp. 2, activity was monitored in animals receiving counterbalanced i.p. injections of devazepide (0.1 mg/kg) or vehicle during their LP followed 30 min later by i.p. injection of either amphetamine (AMPH; 1.0 mg/kg) or saline. In exp. 3, animals were either sensitized to the locomotor activating effects of AMPH via injection of AMPH (1.5 mg/kg) in their home cage once per day for seven days or were given saline injections. Following a 10 day withdrawal period, AMPH-pretreated animals received an i.p. injection of devazepide (0.001, 0.01, or 0.1 mg/kg) or vehicle during their LP, followed 30 min later by i.p. AMPH challenge (0.75 mg/kg). Saline-pretreated controls were given vehicle injections followed by AMPH.

Results from exp. 1 revealed that while locomotor activity was greater during DP than LP testing, devazepide had no effect in either condition at any dose tested. Results from exp. 2 demonstrated that devazepide had no effect on locomotion induced by acute AMPH administration, and that this negative finding was independent of baseline exploration levels. Exp. 3 showed that AMPH-sensitized animals demonstrated a greater locomotor response to AMPH challenge than saline-pretreated controls, and that this augmented response was attenuated by devazepide at 0.1 mg/kg, but not at 0.001 or 0.01 mg/kg. Taken together, these data suggest that endogenous CCK, acting at the CCK-A receptor, modulates the expression of DA-mediated behaviour under conditions of elevated DAergic activity.